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Kepone (Trade Name) Effects on Development of 'Callinectes sapidus' and 'Rhithropanopeus harrisii'

Duke Univ, Beaufort, NC Marine Lab

Prepared for

Environmental Protection Agency, Gulf Breeze, FL

Sep 79

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Gulf Breeze FL 32561

EPA-600/3-79-104
September 1979

PB80-134349

Research and Development

EPA

Kepone® Effects on Development of *Callinectes sapidus* and *Rhithropanopeus harrisii*

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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO. PB 80 134349
4. TITLE AND SUBTITLE KEPONE[R] EFFECTS ON DEVELOPMENT OF CALLINECTES SAPIDUS AND RHITHROPANOPEUS HARRISII		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) C.G. Bookhout, J.D. Costlow, Jr., R.J. Monroe* *North Carolina State University		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Duke University Marine Laboratory Beaufort, N.C. 28516		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO. R803838
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Gulf Breeze, Florida 32561		13. TYPE OF REPORT AND PERIOD COVERED Final Scientific Report 14. SPONSORING AGENCY CODE EPA/600/4
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Laboratory experiments were conducted to determine the effect of Kepone on the development of Callinectes sapidus from the time of hatching until the 1st crab stage was reached. For comparison, similar investigations were made to ascertain the effects of Kepone on larval development of Rhithropanopeus harrisii.

Of the concentrations tested, 35, 50, 65 and 80 ppb Kepone were found to be sublethal and 95, 110 and 125 ppb Kepone were acutely toxic to R. harrisii larvae; whereas 0.1, 0.5 and 0.75 were sublethal and 1.0 ppb Kepone were acutely toxic to C. sapidus larvae. The duration of zoeal development and total time from hatching to 1st crab was in general prolonged with concentration in R. harrisii; whereas, in C. sapidus, no significant relationship could be detected between Kepone concentration and duration of zoeal development, but there was a significant relationship to 1st crab. The developmental stages in which the larvae are particularly sensitive differ in the two species.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Insecticides Bioassay Crustacea Crabs Kepone	Insecticide toxicity Kepone Blue crabs Mud crabs Larval development	

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	47
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE PC A03 MF A01

Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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EPA-600.3-79-104
September 1979

KEPONE® EFFECTS ON DEVELOPMENT OF *CALLINECTES SAPIDUS* AND *RHITHROPANOPEUS HARISII*

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The EPA Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms
- the effects of toxic organics on ecosystem processes and components
- the significance of chemical carcinogens in the estuarine and marine environments

The investigation described in this report sought to determine the sublethal and acutely toxic effects of the pesticide Kepone® on the mud crab, Rhithorpanopeus harrisii (Gould), and the blue crab, Callinectes sapidus Rathbun, during larval development and early crab stages. These data should contribute to our knowledge of Kepone® concentrations affecting larval survival, length of larval development, molting, and ultimately the population biology of the two species.

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ABSTRACT

Laboratory experiments were conducted to determine the effect of Kepone on the development of Callinectes sapidus from the time of hatching until the 1st crab stage. For comparison, similar investigations were made to ascertain the effects of Kepone on larval development of Rhithropanopeus harrisii.

Of the concentrations tested, 35, 50, 65, and 80 parts per billion (ppb) Kepone were found to be sublethal and 95, 110, and 125 ppb Kepone were acutely toxic to R. harrisii larvae; whereas 0.1, 0.5, and 0.75 were sublethal and 1.0 ppb Kepone was acutely toxic to C. sapidus larvae. The duration of zoeal development and total time from hatching to 1st crab were in general prolonged with concentration in R. harrisii; whereas, in C. sapidus, no significant relationship could be detected between Kepone concentration and duration of zoeal development, but there was a significant relationship to 1st crab. The developmental stages in which the larvae are particularly sensitive differ in the two species.

This report was submitted in fulfillment of Grant No. R803838 by Duke University under the sponsorship of the Environmental Protection Agency. This report covers the period August 30, 1977, to May 31, 1979, and work was completed as of July 30, 1979.

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ACKNOWLEDGMENTS

The help of Dr. Adam Zsolnay, Chemist at Duke University Marine Laboratory, is gratefully acknowledged.

The technical assistance of Mrs. Sally Herring and Mr. Joe Goy and the untiring efforts of Mrs. Norma Jean Buck in typing the manuscript are greatly appreciated.

SECTION 1

INTRODUCTION

Kepone® (decachlorooctahydro-1,3,4-2H-cyclobutu [cd] pentalene-2-one) was developed by the Allied Chemical Company in the early 1950's (EPA, 1975). From 1966 to 1973 it was manufactured at its plant at Hopewell, Virginia. In 1974 production was turned over to Life Science Products Co., a firm established in Hopewell by two former Allied employees. Allied furnished the raw materials and bought the finished product. Life Science Products Co. operated until July 1975 when it was closed under orders of the Virginia Health Department because tests showed that Life Science workers had been poisoned and the lower part of the James River was heavily contaminated with Kepone.

Kepone, when added to bait or other inert material, is used to kill cockroaches and ants, and to control potato and banana pests. It is used in over 50 commerical formulations, but is most commonly used to control banana pests in Latin America (EPA, 1975).

The contamination of sediment, water and biota of the freshwater and estuarine portions of the James River raised questions concerning the acute and chronic effects of Kepone on aquatic life in the river and the potential danger to humans eating contaminated animals (Hansen et al., 1976).

Kepone is structurally similar to the insecticide, mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene). Kepone, like mirex, is very stable. It is readily taken from water by marine organisms (Walsh et al., 1977; Schimmel and Wilson, 1977; Nimmo et al., 1977; and Hansen et al., 1977), and is passed through the food chain (Bahner et al., 1977). Oysters (Crassostrea virginica), mysids (Mysidopsis bahia), grass shrimp (Palaemonetes pugio), and spot (Leiostomus xanthurus) bioconcentrate Kepone from concentrations as low as 0.25 µg/l seawater. In these species bioconcentration factors range from 2,300 to 13,500 in long-term flow-through bioassays. Depuration varies among species that have been tested. In oysters it is rapid with no Kepone detected in tissue after 7 to 20 days. The rate of depuration in grass shrimp and fish is slower. In these animals Kepone concentrations are reduced by 30 to 50% in 24 to 28 days. Approximately 3000 times as much Kepone in food as in water is needed to reach a similar concentration in 28 to 30 days in spot (Bahner et al., 1977).

There have been few reports on chronic effects of Kepone on the early development and/or larval development of estuarine animals. Hansen et al.

(1977) studied the chronic effects of Kepone on embryo, fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus). The only life history toxicity tests on a malacostracan crustacean have been made on mysids, Mysidopsis bahia (Nimmo et al., 1977). In a 19-day bioassay survival was reduced from 84.4% in 0.39 µg/l to 0% in 8.7 µg/l Kepone. Sublethal effects included reduction in young, delay in formation of brood pouches, delay in release of young and reduced growth.

As far as known, there have been no publications on the effects of Kepone on the complete development of any crab. The objectives of the current investigation, therefore, are to determine the limits of concentration of Kepone within which the mud crab, Rhithropanopeus harrisii (Gould) and the blue crab, Callinectes sapidus Rathbun, can be reared from the time of hatching until the 1st crab is reached, and to ascertain the sublethal and acutely toxic concentrations of Kepone. From these studies, it should be possible to determine if there are sublethal effects, and if there are one or more larval stages of development which are particularly sensitive. A final objective is to determine if there is a statistically significant effect of Kepone on survival to megalopa and to first crab, duration from hatching to megalopa and to 1st crab, and cumulative mortality by stages of development of R. harrisii and C. sapidus.

SECTION 2

SUMMARY AND CONCLUSIONS

1. Differential survival of Rhithropanopeus harrisii from hatching to 1st crab stage occurred in a range of concentrations of Kepone from 35 to 125 ppb. Concentrations were sublethal from 35 to 80 ppb and acutely toxic from 95 to 125 ppb. Statistical analysis indicated a 15% decrease in survival for each 10 ppb increase in Kepone in the range of 30 to 100 ppb Kepone.

2. Differential survival of Callinectes sapidus from hatching to 1st crab stage occurred in a range of concentrations of Kepone from 0.1 to 1.0 ppb. Concentrations were sublethal from 0.1 to 0.75 ppb and were acutely toxic at 1.0 ppb. Statistical analysis indicated a decrease of 2.2% in survival for each 0.1 ppb increase in Kepone in the range of 0.1 to 0.8 ppb Kepone.

3. In R. harrisii, the time from hatching to megalopa and hatching to 1st crab is generally prolonged with each concentration of Kepone from 35 to 100 ppb, primarily due to prolongation of zoeal development. Statistical analysis indicates that, for every 10 ppb Kepone added, duration from hatching to 1st crab stage is increased by 0.391 $\pm$ 0.043 days.

4. In C. sapidus, no significant relation could be detected between Kepone concentration and duration of zoeal development; however, a significant linear relationship was established between Kepone levels and duration to 1st crab. For each increase of 0.1 ppb, the duration from hatching to 1st crab is prolonged by 0.38 $\pm$ 0.10 days.

5. The 1st and 2nd zoeal stages of R. harrisii were the most sensitive developmental stages to Kepone, and thereafter there was less mortality among the remaining resistant larvae.

6. The 1st zoeal stage of C. sapidus was not sensitive, statistically, to any concentration of Kepone tested. In zoeal stages II, III, and IV, there were significant increases in mortality over the previous stage in all concentrations and in the control. There was a trend of increasing mortality with concentration, but only in the highest concentration (1.0 ppb) was the mortality significantly greater than in the control.

SECTION 3

RECOMMENDATIONS

1. It is important to determine the effects of Kepone on the larval development of R. harrisii in the middle estuary of the James and on the development of C. sapidus in the lower Chesapeake Bay, because larvae are a natural component of the food chain in these waters and any detrimental effect of Kepone on developmental stages will reduce the adult population. Therefore, larvae should not be disregarded when safe water standards for estuaries are set.

2. To determine the effect of Kepone on the development of crabs in the field, there should be an annual program of monitoring sediment, water and zooplankton for Kepone. Residue analyses should be made of adult R. harrisii and C. sapidus, their eggs and larvae at periodic intervals throughout the breeding season.

3. With reliable field data available, laboratory experiments should be designed to simulate field conditions. The concentration of Kepone in eggs of each species could be obtained from field studies, but the additional uptake from seawater and from food, with concentrations similar to those in the field, can be determined best through laboratory studies. A correlation of Kepone residues with mortality and exposure time should provide an indication of the effect of Kepone on larval development of each species in the field.

4. The current report outlined the effects of Kepone on the development of crabs when larvae were reared under optimum temperature and salinity conditions. Further investigation should be made to determine the effect of Kepone on the larval development of crabs when reared in a range of salinities and temperatures. Since there is some evidence that larval survival of C. sapidus is better in the early part of the breeding season and that total duration from hatching to 1st crab is shorter than in the latter part of the breeding season, further experiments should be done to determine if the larvae are more resistant to Kepone in the early part of the breeding season than the later part.

5. Residue analyses of larvae reared in different concentrations of Kepone for varying lengths of time were not a part of the current project, but they would have been desirable. From past experience, we found it to be a time-consuming task to obtain 0.20 g of larvae, wet weight, in low concentrations of a chlorinated hydrocarbon insecticide and impossible to obtain enough late-stage larvae in high concentrations to make a residue anlaysis. Methods should be developed, therefore,

to make accurate residue analyses of insecticides from samples of larvae weighing less than 0.20 g wet weight. For a stable compound, such as Kepone, it would seem useful to utilize radiolabeled material for such small samples, especially in a static test.

SECTION 4

MATERIALS AND METHODS

Preparation

Preliminary experiments were conducted to determine the range of concentrations to use in definitive chronic experiments on the effect of Kepone on the development of the mud crab, Rhithropanopeus harrisii (Gould), and the blue crab, Callinectes sapidus Rathbun.

Pesticide grade acetone was used as a carrier for Kepone. Acetone control for experiments with Kepone was prepared by adding 1 ml of full strength acetone to 999 ml of 20°/oo filtered seawater for R. harrisii larvae and 30°/oo filtered seawater for C. sapidus larvae to give a final concentration of 1.0°/oo (parts per thousand).

Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cylobuta[cd] pentalene 2-one) was purchased from Chemical Service, West Chester, PA, as 99% pure, but analysis at Environmental Research Laboratory, Gulf Breeze, Florida, indicated 88% purity after exposure to air (personal communication). Dr. Adam Zsolnay, chemist at Duke University Marine Laboratory, prepared stock solutions by dissolving a known weight of Kepone in pesticide analytical grade acetone, and different concentrations were made up from this stock solution.

For experiments on the effect of Kepone on the development of R. harrisii, 1 ml of stock solutions of 35.0 parts per million (ppm) (mg/l), 50.0 ppm, 65.0 ppm, 80.0 ppm, 95.0 ppm, 110.0 ppm and 125.0 ppm was added to 999 ml of 20°/oo filtered seawater daily to give final concentrations of 35.0 parts per billion (ppb) (µg/l) to 125.0 ppb Kepone.

For experiments on the effect of Kepone on the development of C. sapidus one ml of stock solutions of 0.1 ppm, 0.5 ppm, 0.75 ppm and 1.0 ppm was added to 999 ml of 30°/oo filtered seawater daily to give final concentrations of 0.1 ppb to 1.0 ppb.

Source of mother crabs and hatching of eggs

Ovigerous R. harrisii, small mud crabs belonging to the family Xanthidae, were collected in the vicinity of Fort Pierce, Florida, in February, April, and May 1978. They were shipped to Beaufort, North Carolina, by air freight. Upon arrival at Duke University Marine Laboratory

(DUML), Beaufort, North Carolina, each mud crab was placed in a large glass finger bowl (19.4 cm d) containing 20 °/oo filtered seawater, the salinity to be used during the rearing experiments with Kepone. The ovigerous crabs were maintained in a constant temperature cabinet at 25 °C and with a light regime of 12h light and 12h darkness until hatching of larvae occurred.

Ovigerous C. sapidus, commerical blue crabs belonging to family Portunidae, were collected off Beaufort Inlet, North Carolina, in July 1978. The crabs used had black eggs with a visible heart beat. The method for developing eggs has been described previously (Costlow and Bookhout, 1960; Bookhout and Costlow, 1975).

Rearing of larvae

Five replicate series of R. harrisii larvae and three of C. sapidus were reared in acetone control and different concentrations of Kepone. Each series of larvae was hatched from a different mother crab. Series IX of R. harrisii was designated as Rh IX and the date of hatch was 2-19-78. The dates of the other series are as follows: Rh X, 2-28-78; Rh XIII, 4-9-78; Rh XIV, 4-13-78; and Rh XVII, 5-14-78. Series Cs XII and Cs XIII of C. sapidus were hatched 7-26-78, and Cs XIV, 7-27-78. The methods for rearing larvae of each species in a check series, 10 larvae per finger bowl (8.9 cm diam), and an insecticide, were described previously (Bookhout et al., 1976).

SECTION 5

STATISTICAL ANALYSIS OF LARVAL DEVELOPMENT OF *R. HARRISII* AND *C. SAPIDUS* SUBJECTED TO TREATMENT BY KEPONE

General

Three types of analysis were done on *R. harrisii* and *C. sapidus* data:

(i) Analysis of survival percentages using survival to megalopa and to the first crab stage

(ii) Analysis of duration of molting for the times to megalopa and to the first crab stage. Duration was measured in DAYS and analyzed as such. In addition a new variable was constructed which has units of reciprocal days and is therefore proportional to rate of molting. The exact transform was RATE = 100/DAYS where the constant 100 is a convenient scale factor. In many biological systems where time is a relevant variable, the use of the reciprocal transform often simplifies relationships to experimental factors, and when the variability of the time measurement tends to increase with large values of time, the reciprocal transform usually has nearly constant variance

(iii) Analysis of cumulative mortality by stage of development

All computations were done using the Statistical Analysis System (SAS) (Barr *et al.*, 1976). PROC GLM (The General Linear Models Procedure) with least squares means and standard error options was the primary procedure.

Analyses of survival percentages

The percent survival to megalopa and to first crab was first transformed to the angle Y, such that $\text{Sin}^2 Y = P$ (percent survival) or $Y = \arcsin \sqrt{P} = \sin^{-1} \sqrt{P}$. This transformation tends to stabilize the error variance across the experiment and may help to linearize the response to the several Kepone levels (Snedecor and Cochran, 1967).

The general linear model technique (Searle, 1971) was used to account for the unequal numbers of replicates at each Kepone level. The transformed % survival, Y, was regressed on the several levels of Kepone, the regression coefficient and its standard error computed, and the deviations from linearity tested for significant departure from a straight line relationship. The implications of this approach is (i) that if a functional relationship can be established between a response, say Y, and the concentration of Kepone,

then the regression coefficient expressed in degrees/ppb Kepone is the most efficient expression of that relationship, and (ii) if significant departures from linearity are observed, then the simple model either must be restricted to that range of concentrations in which the relationship is linear, or discarded in favor of a more complicated model that contains terms to allow for the nonlinearity of response. Linearity in the transformed survival implies nonlinearity in the relationship between % survival and Kepone concentration; but approximate expressions may be obtained for the rate of change of % survival with respect to concentration by restricting the range of concentrations to one in which the derivatives are nearly constant. More formally we have:

Observed, $Y = a + bX$, b measured in degrees/ppb.

Wanted, dP/dX, where $Y = \arcsin \sqrt{P}$,

and $$dP/dX = dY/dX \cdot dP/dY = \frac{2b\sqrt{P(1 - P)}}{57.3}, \ ^{o}/o/ppb.$$

Thus, when the quantity $\sqrt{P(1 - P)}$ is approximately constant for a range of P from 0.10 to 0.90, we can also express the results in the original, and more familiar, scale of percent. The constant 57.3 converts angles in degrees to angles in radians.

It is appropriate to mention at this point that a "significant" regression coefficient is one which has been judged not zero and implies by itself nothing about the biological importance or implications.

Analysis of duration/rate

The variables analyzed and their abbreviations are:

DZ = DAYS to megalopa

RZ = 100/DAYS, the variable proportional to molting rate measured in reciprocal DAYS x 100.

DC = DAYS to first crab

RC = 100/DC analogous to RZ.

The general linear model technique was employed to estimate the regression coefficients of each of the above variables on the Kepone concentration, adjusting for unequal replication. The departure from linearity was tested at the same time.

The regression coefficient has units of DAYS/ppb (or 100/DAYS/ppb) and is said to measure a significant effect of Kepone if it is more than twice its estimated standard error. If, in addition, there is no significant departure from linearity, it is then the single best expression of the relationship between molting time (or rate) and Kepone concentration.

As noted earlier, the rate variable is often linear with, say, concentration if duration in DAYS is not. If best linearity is found in Rate (RZ), then the equivalent value for duration would be obtained by

$$\text{DAYS/ppb} = b\left(\frac{-100}{(RZ)^2}\right) \text{ where}$$

b = linear regression coefficient of RZ on Concentration (ppb).

This value is clearly not now a constant but an approximate figure may be obtained by using the average value of RZ for the several Kepone levels involved.

Analysis of cumulative mortality

The percent cumulative mortality by stage of development was also transformed to the angular scale for analysis. The analysis of these data, however, had to account for the additional factors STAGE and the STAGE* CONCENTRATION interaction following the technique for a split-plot analysis of variance (Cochran and Cox, 1957). The introduction of another level of classification required that the error structure be subdivided into two components usually designated as the sub-plot error (E_b) and the whole-plot error (E_a).

Comparisons of mean mortalities among STAGES at the same concentration of Kepone were made using standard errors computed from E_b only. Comparisons of mean mortalities among concentration levels at the same STAGE or at different STAGES were made using a weighted standard error involving both estimates of error. Specifically the weighted mean square error,

$$E_w = \frac{(c-1)E_b + E_a}{c},$$

where c = number of levels of concentration. Since $E_a \geq E_b$ then also $E_w \geq E_b$.

All tests of differences between mean mortalities among STAGES and CONCENTRATIONS were completed in the transformed scale but the results were reported in the original percentage scale.

SECTION 6

RESULTS

EFFECTS OF KEPONE ON DEVELOPMENT OF RHITHROPANOPEUS HARRISII

Survival

The percent survival to megalopa and to the first crab stage is given for five series in TABLE 1. The average percent survival of all series reared in various media is given in TABLE 2. Survival of larvae from hatching to megalopa and to first crab stage in five replicate series reared in acetone control only varied from 98 to 100% and 94 to 100%, respectively (TABLE 1). There was differential survival in concentrations of Kepone from 35 ppb to 125 ppb (TABLE 2).

Statistical analysis of R. harrisii survival

The results shown in Figure 1 indicate the linear relationship between the concentration of Kepone and survival to the megalopa, and to 1st crab stages. Statistical analyses indicated that:

(i) the dose-response relationship is linear only in the range of 35-110 ppb Kepone. When the control and 125 ppb survivals are included, the deviations from linearity become significant.

(ii) In the range of 35-100 ppb no significant differences in the dose-response relationship exist between survival from hatching to megalopa and survival from hatching to first crab either with respect to the slope of the line or the level of survival. This result suggests that survival of megalopa is not affected much by the Kepone levels in this range. The results in Figure 1 are the combined data.

$b = -0.9854 \pm 0.0557$ degrees decrease in survival for the increase of 1 ppb Kepone.

The b-value here is not easily converted back to the original units of percent survival, but would correspond approximately to a 15% decrease in survival for each 10 ppb Kepone in the range of 30 to 100 ppb concentration.

TABLE 1. EFFECTS OF KEPONE ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT IN *Rhithropanopeus harrisii* IX, X, XIII, XIV AND XVII

Culture media Salinity 20°/oo Temp. 25°C	Initial No. of larvae per per series	% Survival to		Mean duration of development in days		
		Megalopa	1st Crab	Zoea	Megalopa	Hatch to 1st Crab
Acetone Control	Rh IX-50	98	94	11.20	5.74	16.94
	Rh X-50	100	98	9.88	6.12	16.00
	Rh XIII-50	98	94	10.78	4.82	15.79
	Rh XIV-50	100	100	10.10	5.56	15.68
	Rh XVII-50	100	98	11.02	6.29	17.30
Kepone 35 ppb	Rh XIII-50	90	80	12.31	5.90	18.13
	Rh XIV-50	92	88	12.04	5.55	17.61
	Rh XVII-50	94	90	13.04	5.73	19.20
Kepone 50 ppb	Rh X-50	78	64	11.64	5.34	16.91
	Rh XVII-50	70	64	13.91	6.00	19.90
Kepone 65 ppb	Rh IX-50	48	32	12.63	5.94	18.56
	Rh X-50	80	68	11.93	5.91	17.50
	Rh XIII-50	86	74	13.33	6.76	20.10
	Rh XIV-50	98	94	11.63	6.02	17.35
	Rh XVII-50	48	38	15.00	7.21	21.90
Kepone 80 ppb	Rh IX-50	26	10	14.77	6.60	20.80
	Rh X-50	44	26	12.59	5.46	17.77
	Rh XVII-50	4	4	15.50	6.00	21.50
Kepone 95 ppb	Rh IX-50	6	2	14.00	6.00	19.00
	Rh X-50	20	16	13.10	6.25	18.75
	Rh XIII-50	0	0	-	-	-
	Rh XIV-50	0	0	-	-	-
	Rh XVII-50	4	4	17.50	7.50	25.00
Kepone 110 ppb	Rh IX-50	6	2	14.33	7.00	20.00
	Rh X-50	12	2	15.50	6.00	19.00
	Rh XIII-50	0	0	-	-	-
	Rh XIV-50	0	0	-	-	-
Kepone 125 ppb	Rh IX-50	0	0	-	-	-
	Rh X-50	4	2	14.50	6.00	19.00
	Rh XIII-50	0	0	-	-	-
	Rh XIV-50	0	0	-	-	-

TABLE 2. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF *R. harrisii* REARED IN ACETONE CONTROL AND DIFFERENT CONCENTRATIONS OF KEPONE

Culture media Salinity 20°/oo Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean duration of development in days		
		Megalopa	1st Crab	Zoea	Megalopa	Hatch to 1st crab
Acetone Control	Rh IX-50 Rh X-50 Rh XIII-50 Rh XIV-50 Rh XVII-50	99.2	96.8	10.59	5.70	16.34
Kepone 35 ppb	Rh XIII-50 Rh XIV-50 Rh XVII-50	92	86	12.59	5.72	18.33
Kepone 50 ppb	Rh X-50 Rh XVII-50	74	64	12.72	5.67	18.41
Kepone 65 ppb	Rh IX-50 Rh X-50 Rh XIII-50 Rh XIV-50 Rh XVII-50	72	63.2	12.69	6.32	18.75
Kepone 80 ppb	Rh IX-50 Rh X-50 Rh XVII-50	26	13.3	13.51	5.80	18.90
Kepone 95 ppb	Rh IX-50 Rh X-50 Rh XIII-50 Rh XIV-50 Rh XVII-50	6	4.4	13.87	6.45	19.91
Kepone 110 ppb	Rh IX-50 Rh X-50 Rh XIII-50 Rh XIV-50	4.5	1.0	15.11	6.50	19.50
Kepone 125 ppb	Rh IX-50 Rh X-50 Rh XIII-50 Rh XIV-50	1	0.5			

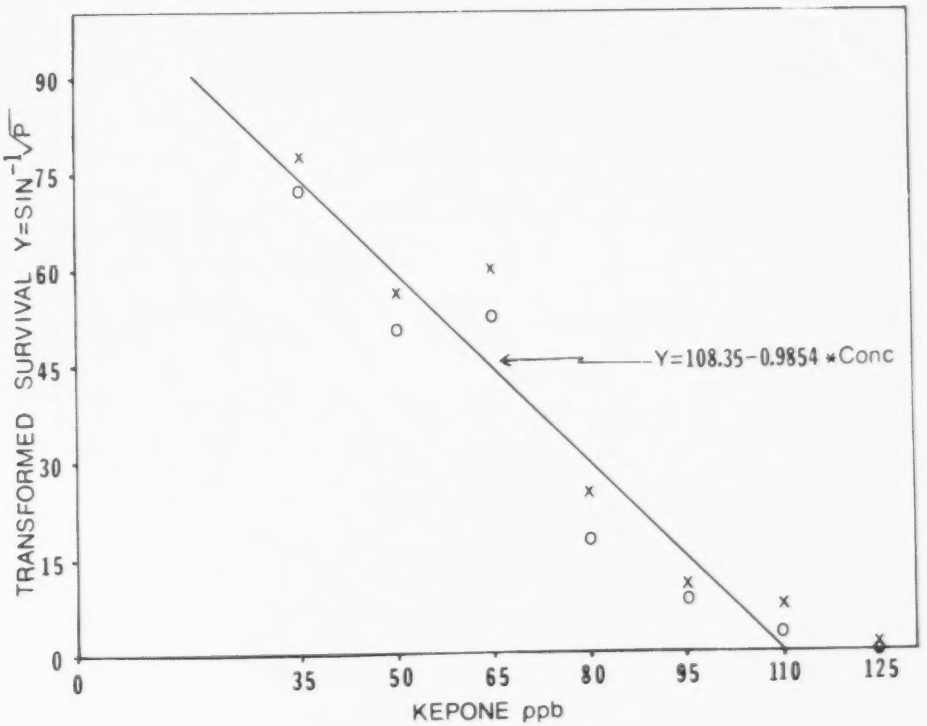

Figure 1. Effect of Kepone concentration on survival of R. harrisii.
Hatch to megalopa x ——— x
Hatch to 1st crab o ——— o

Duration

TABLE 1 gives the mean duration in days of zoeal and megalopa development and the mean time in days from hatching to the 1st crab stage for each series reared in acetone control and in different concentrations of Kepone. TABLE 2 lists the mean duration of development in days for all larvae reared in all series. Each table shows that the duration of zoeal development is in general prolonged with each concentration from 35 ppb to 110 ppb Kepone. Since the mean duration of megalopa development was somewhat similar in these concentrations, the total time from hatching to the 1st crab stage was prolonged with each higher concentration primarily due to the effect of Kepone on zoeal development.

Statistical analysis of R. harrisii duration

1. Significant linear regressions of both DZ (days to megalopa) and DC (days to 1st crab) upon Kepone levels were found with no significant deviations from linearity. The most compact summary is in these equations:

   DZ = 10.5 + 0.0423 * CONC

   DC = 16.5 + 0.0359 * CONC

   where CONC is in ppb of Kepone. These results are shown in Figure 2. The regression coefficients may be interpreted as follows:

   for DZ: 0.0423 $\pm$ 0.0051 days increase in duration of zoeal development for each ppb added Kepone

   for DC: 0.0359 $\pm$ 0.0069 days increase in total duration time for each ppb added Kepone.

   These increases in duration can be scaled up, for example to 10 ppb, by multiplication, i.e., 0.423 $\pm$ 0.051 days increase for each 10 ppb added Kepone.

The test of significance of the difference between the two slopes is also not significant which suggests that a pooled estimate can be used to characterize the concentration effect on duration. This estimate is

b = 0.0391 $\pm$ 0.0043 DAYS/ppb Kepone

2. Nearly analogous results were obtained when RATE = 100/DAYS was used as the dependent variable:

   RZ = 9.31 - 0.0251 * CONC

   RC = 6.01 - 0.0100 * CONC

   These results are shown in Figure 3:

   b(RZ) = -0.0251 $\pm$ 0.0024 reciprocal days decrease in RATE for each ppb increase in Kepone and

   b(RC) = -0.0100 $\pm$ 0.0015 reciprocal days decrease in RATE for each ppb increase in Kepone.

   Now these two coefficients do differ significantly from each other which may suggest that the Kepone effect is greater on zoeal stage than on the megalopa.

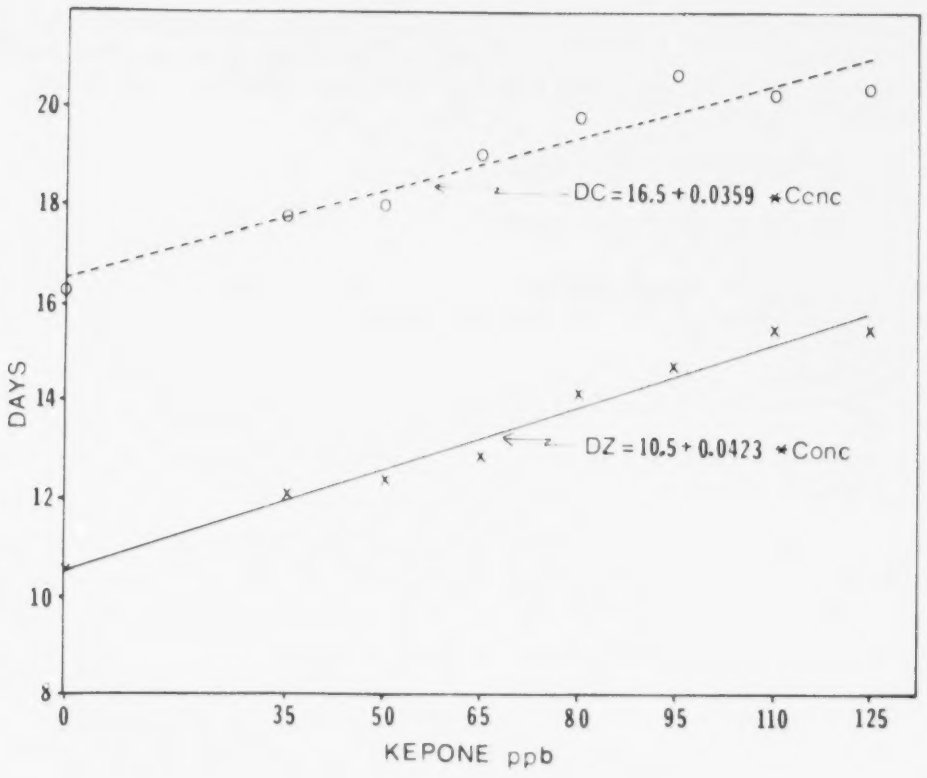

Figure 2. Duration zoeal development (DZ) and hatch to crab (DC) in R. harrisii vs concentration of Kepone.
DZ: x ——— x Hatch to megalopa
DC: o ----- o Hatch to 1st crab

Mortality

Rhithropanopeus harrisii passes through four zoeal stages and a megalopa stage before molting into a 1st crab stage. In an effort to determine if larvae in one or more stages of development were particularly sensitive to different concentrations of Kepone, a record of deaths by stage was made for each of the five series of larvae (Table 3).

TABLE 3. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF *R. harrisii*

Media	Series	Zoeal Stages				Megalopa	Total
		I	II	III	IV		
Acetone Control	Rh IX	0	2	0	0	4	6
	Rh X	0	0	0	0	2	2
	Rh XIII	2	0	0	0	4	6
	Rh XIV	0	0	0	0	0	0
	Rh XVII	0	0	0	0	2	2
Kepone 35 ppb	Rh XIII	2	4	0	4	10	20
	Rh XIV	8	0	0	0	4	12
	Rh XVII	0	6	0	0	4	10
Kepone 50 ppb	Rh X	0	8	0	14	14	36
	Rh XVII	20	10	0	0	6	36
Kepone 65 ppb	Rh IX	4	32	4	12	16	68
	Rh X	0	4	4	12	12	32
	Rh XIII	0	0	0	14	12	26
	Rh XIV	0	0	0	2	4	6
	Rh XVII	14	34	0	4	10	62
Kepone 80 ppb	Rh IX	2	52	8	12	16	92
	Rh X	16	20	8	12	18	74
	Rh XVII	36	52	8	0	0	96
Kepone 95 ppb	Rh IX	34	46	6	8	4	98
	Rh X	44	22	10	4	4	84
	Rh XIII	100	-	-	-	-	100
	Rh XIV	98	0	0	2	-	100
	Rh XVII	28	60	8	0	0	96
Kepone 110 ppb	Rh IX	32	58	2	2	4	98
	Rh X	48	24	8	8	10	98
	Rh XIII	100	-	-	-	-	100
	Rh XIV	100	-	-	-	-	100
Kepone 125 ppb	Rh IX	34	60	4	2	0	100
	Rh X	66	20	8	2	2	98
	Rh XIII	92	8	-	-	-	100
	Rh XIV	96	4	-	-	-	100

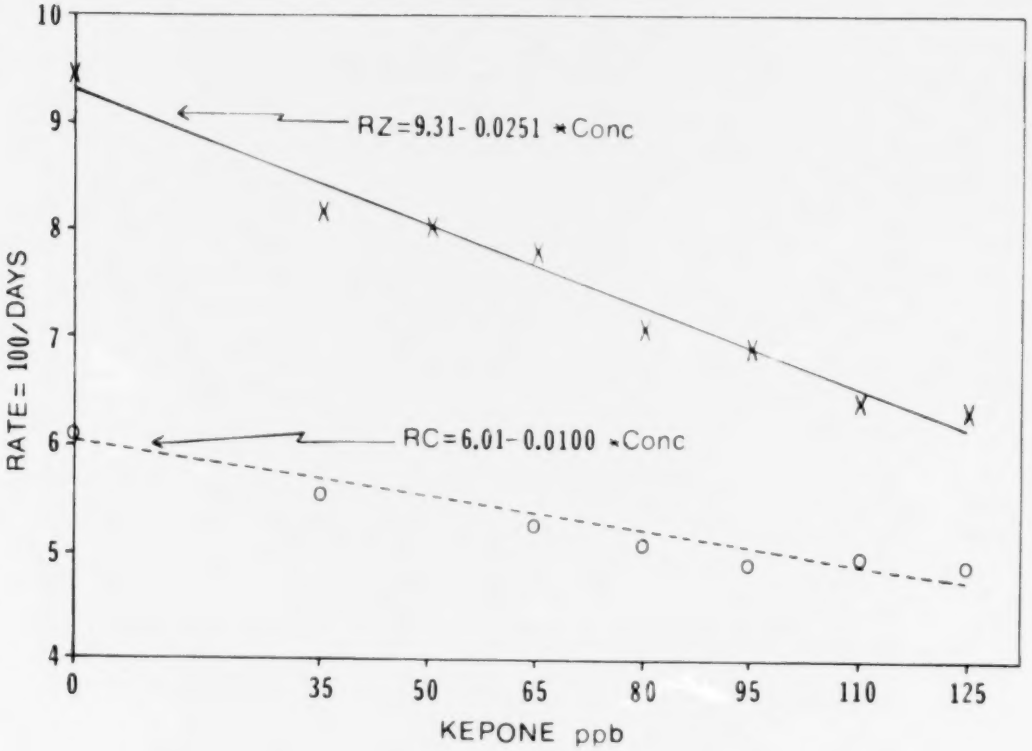

Figure 3. Effect of Kepone on rate of molting from hatch to megalopa and hatch to crab in R. harrisii.
RZ: x ——— x Hatch to megalopa
RC: o ----- o Hatch to 1st crab

Statistical analysis of R. harrisii cumulative mortality by stages

The results given in Figure 4 show the effect of Kepone levels on the mortality at each stage of development. The percent mortalities on the graph were obtained from the means of the transformed variable adjusted for the unequal number of replications for several treatments.

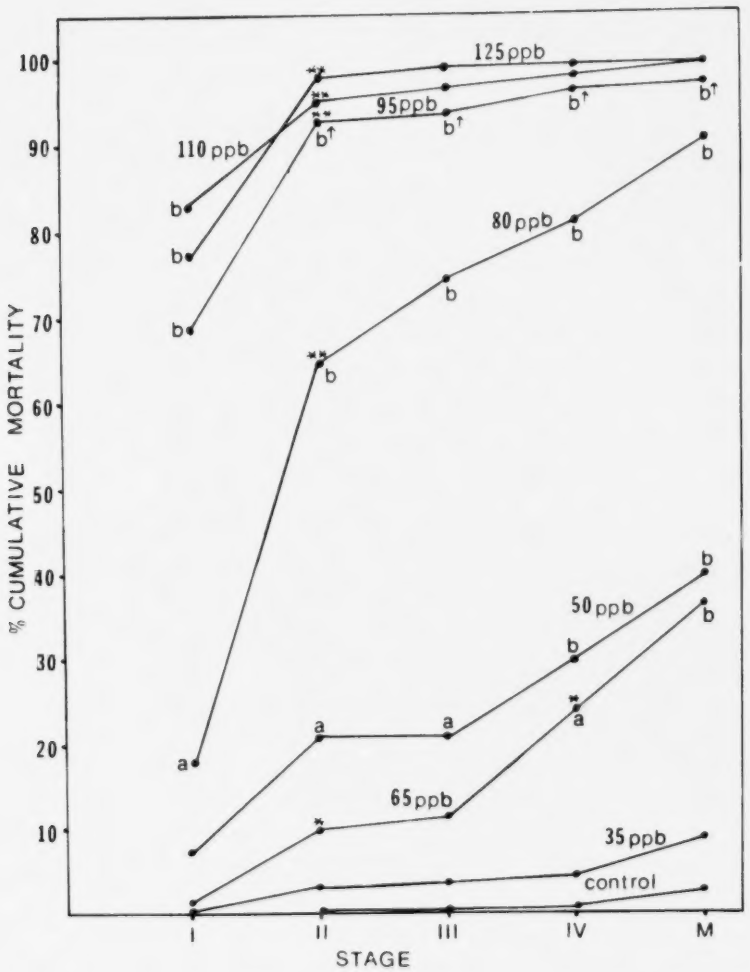

Figure 4. Effect of Kepone on mortality by stages of development of R. harrisii.
a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

EFFECTS OF KEPONE ON DEVELOPMENT OF CALLINECTES SAPIDUS

Survival

The percent survival to megalopa and to the 1st crab stage is given for three series in TABLE 4. The average percent survival of all series

TABLE 4. EFFECTS OF KEPONE ON PERCENT SURVIVAL AND DURATION IN DAYS THROUGH ZOEAL AND MEGALOPA DEVELOPMENT OF Callinectes sapidus XII, XIII and XIV

Culture media Salinity 30°/oo Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean duration of development in days		
		Megalopa	1st Crab	Zoea	Megalopa	Hatching to 1st Crab
Acetone Control	Cs XII-50	30	20	44.8	9.6	54.3
	Cs XIII-50	32	26	40.4	9.7	50.4
	Cs XIV-50	24	22	46.9	11.1	57.0
Kepone 0.10 ppb	Cs XII-50	34	32	42.2	9.9	52.3
	Cs XIII-50	30	28	41.7	9.4	51.1
	Cs XIV-50	4	4	50.0	11.0	60.1
Kepone 0.50 ppb	Cs XII-50	38	28	44.4	9.9	53.4
	Cs XIII-50	10	10	43.0	8.8	51.8
	Cs XIV-50	10	8	49.2	13.5	59.8
Kepone 0.75 ppb	Cs XII-50	38	24	47.2	9.8	54.8
	Cs XIII-50	10	8	41.0	10.0	51.5
	Cs XIV-50	6	6	48.0	13.7	61.7
Kepone 1.0 ppb	Cs XII-50	14	14	46.3	10.4	57.1
	Cs XIII-50	0	0	-	-	-
	Cs XIV-50	0	0	-	-	-

of larvae reared in acetone control and in 0.1 ppb, 0.5 ppb, 0.75 ppb and 1.0 ppb is given in TABLE 5. There is differential survival with increase in concentration to megalopa and to first crab.

Statistical analysis of C. sapidus survival

As in R. harrisii, a significant linear decrease in survival with Kepone concentration was detected for both zoeal and megalopa survival, and there was no detectable difference between slopes nor between average survival. The combined results are presented in Figure 5 which shows the best prediction equation for the ranges of concentration given.

Again the regression coefficient

$b = -18.5942 \pm 3.2397$ degrees decrease for each ppb increase

i.e., -1.859 ± 0.324 degrees for each 0.1 ppb increase

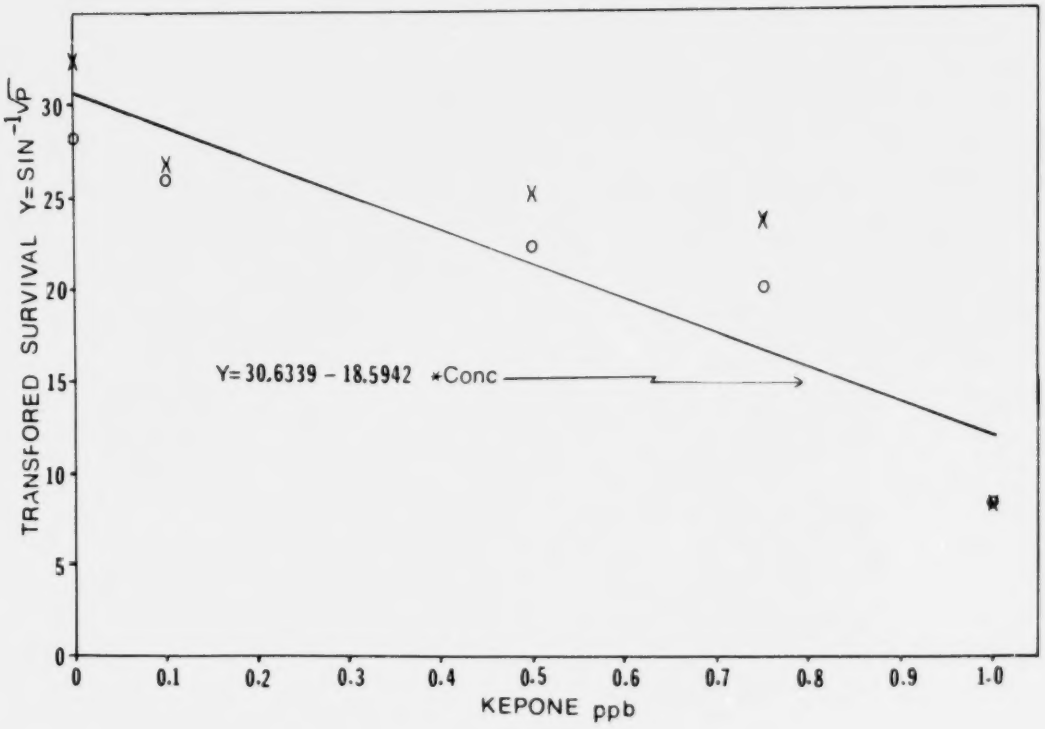

Figure 5. Effect of Kepone concentration on survival of C. sapidus.
Hatch to megalopa x —— x
Hatch to 1st crab o —— o

cannot be easily interpreted in terms of percent survival. However, in the range of 0.1 to 0.8 ppb of Kepone concentration, the decrease of survival is approximately 2.2% for each 0.1 ppb increase in Kepone.

Duration

TABLE 4 gives the mean duration in days of zoeal and megalopa development and the mean time in days from hatching to 1st crab stage for each series reared in acetone control and in 0.1 ppb, 0.5 ppb, 0.75 ppb and 1.0 ppb Kepone. TABLE 5 lists the mean duration of development in days for all larvae reared in all series.

TABLE 5. AVERAGE PERCENT SURVIVAL AND AVERAGE DURATION IN DAYS OF ZOEAL AND MEGALOPA DEVELOPMENT OF Callinectes sapidus REARED IN ACETONE CONTROL AND DIFFERENT CONCENTRATIONS OF KEPONE.

Culture media, Salinity 30°/oo Temp. 25°C	Initial No. of larvae per series	% Survival to		Mean duration of development in days		
		Megalopa	1st Crab	Zoea	Megalopa	Hatching to 1st Crab
Acetone Control	Cs XII-50 Cs XIII-50 Cs XIV-50	28.7	22.7	43.7	10.1	54.7
Kepone 0.10 ppb	Cs XII-50 Cs XIII-50 Cs XIV-50	22.7	21.3	42.47	9.75	52.25
Kepone 0.50 ppb	Cs XII-50 Cs XIII-50 Cs XIV-50	19.3	15.3	44.97	10.26	54.13
Kepone 0.75 ppb	Cs XII-50 Cs XIII-50 Cs XIV-50	18	12.7	46.11	10.42	55.16
Kepone 1.0 ppb	Cs XII-50 Cs XIII-50 Cs XIV-50	4.67	4.67	46.3	10.43	57.14

Statistical analysis of C. sapidus duration/rate

1. No significant relationship could be detected between Kepone concentration and duration of zoeal development (DZ) or rates (RZ).

2. A significant linear relationship was established, however, between Kepone levels and duration to first crab (DC) (Figure 6).

   DC:b = 3.7667 $\pm$ 0.9907 DAYS increase in time from hatch to 1st crab for each ppb increase in Kepone, i.e., 0.38 $\pm$ 0.10 increase for each increase of 0.1 ppb.

   RC:b = -0.1219 $\pm$ 0.0303 reciprocal DAYS decrease for each ppb increase in Kepone, i.e., -0.012 $\pm$ 0.003 reciprocal for each increase of 0.1 ppb.

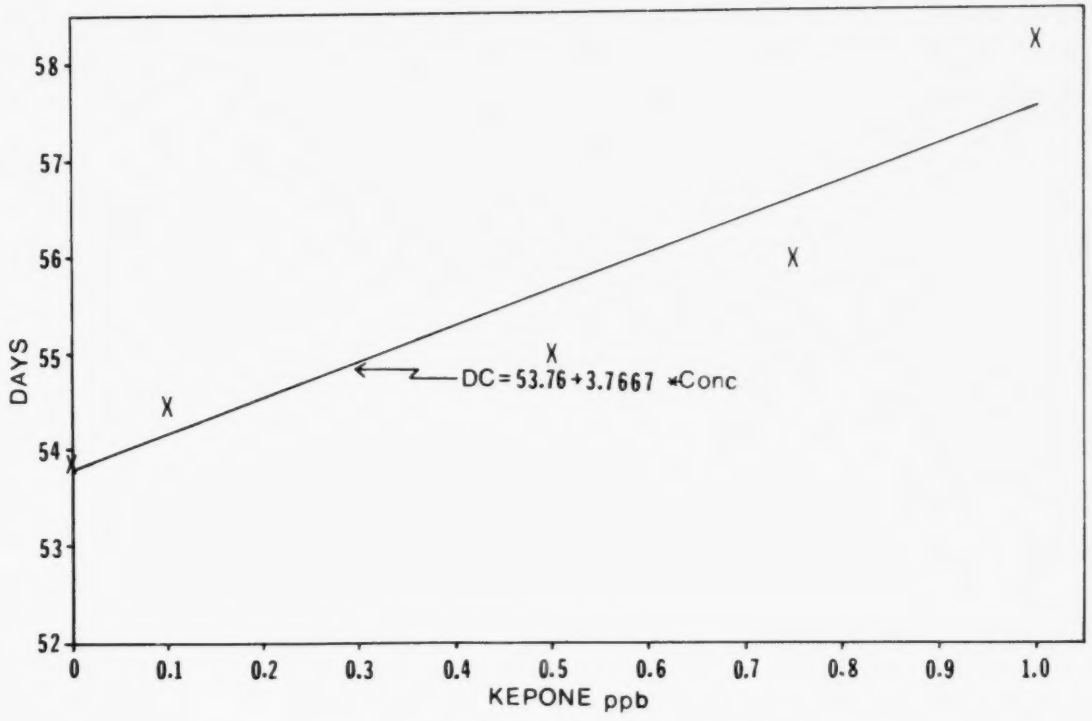

Figure 6. Duration of hatch to 1st crab in C. sapidus vs concentration of Kepone.
DC: Hatch to 1st crab

Mortality

Callinectes sapidus may pass through seven, occasionally eight, zoeal stages before it molts into a megalopa, a ninth stage of development. In these experiments a zoea in an eighth zoeal stage was not observed. In an effort to determine if larvae in one or more of eight developmental stages of C. sapidus were particularly sensitive to different concentrations of Kepone, a record of deaths by stages was made for larvae from each of three crabs, Cs XII - XIV, which had been reared in acetone control and four concentrations of Kepone (TABLE 6).

TABLE 6. PERCENT MORTALITY IN DEVELOPMENTAL STAGES OF C. sapidus

Media	Series	Zoeal Stages							Megalopa	Total
		I	II	III	IV	V	VI	VII		
Acetone	XII	4	30	6	14	6	8	2	10	80
Control	XIII	2	18	16	14	6	0	12	6	74
	XIV	0	10	28	24	6	2	6	2	78
Kepone	XII	8	12	10	22	2	6	6	2	68
0.10 ppb	XIII	2	16	12	10	8	10	12	2	72
	XIV	0	36	28	20	12	0	0	0	96
Kepone	XII	10	12	18	14	8	0	0	10	72
0.50 ppb	XIII	2	38	24	14	6	0	6	0	90
	XIV	0	38	16	26	8	0	2	2	92
Kepone	XII	2	10	32	6	10	0	2	14	76
0.75 ppb	XIII	6	38	22	14	8	0	2	2	92
	XIV	2	38	26	20	6	0	2	0	94
Kepone	XII	8	38	16	14	6	2	2	0	86
1.0 ppb	XIII	10	36	42	8	4	0	0	0	100
	XIV	2	40	22	34	2	0	0	0	100

Statistical analysis of C. sapidus cumulative mortality by stages

The results in Figure 7 show significant increases in mortality at zoeal stages 2, 3, and 4 in all concentrations including the control.

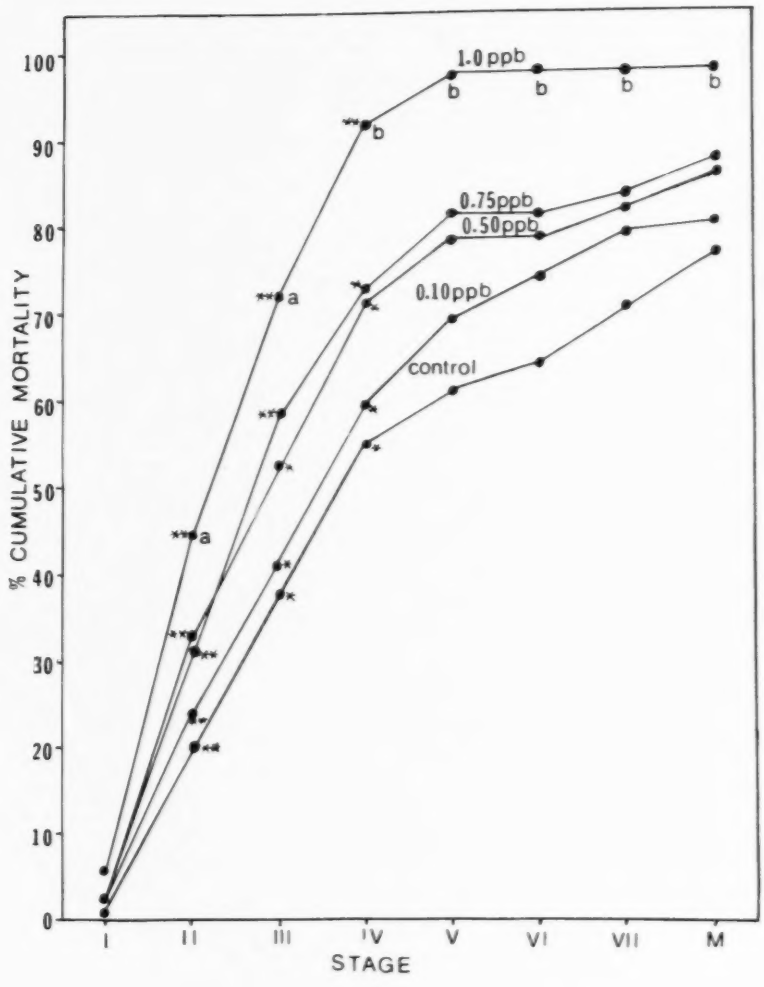

Figure 7. Effect of Kepone on mortality by stages of C sapidus.
a. Significantly different from control (0.05)
b. Significantly different from control (0.01)
*. Significant increase over previous stage (0.05)
**. Significant increase over previous stage (0.01)

SECTION 7

DISCUSSION

Survival

In chronic bioassay studies of the larval development of crabs, sublethal concentrations of a pollutant are arbitrarily defined as those in which there is a reduction in survival with increased concentration of the pollutant and in which at least 10% reach the 1st crab stage. Acutely toxic concentrations are those in which less than 10% of the larvae reach the 1st crab stage (Epifanio, 1971; Bookhout and Costlow, 1975). Since the average survival of R. harrisii from the time of hatching to 1st crab stage was 86% in 35 ppb, 64% in 50 ppb, 63.2% in 65 ppb and 13.3% in 80 ppb Kepone (TABLE 2), these concentrations are considered sublethal. Less than 10% of R. harrisii larvae reached the 1st crab stage in 95, 110 and 125 ppb Kepone (TABLE 2); therefore, these concentrations are considered acutely toxic to R. harrisii larvae. The sublethal concentrations of Kepone to C. sapidus developmental stages were 0.10, 0.50, and 0.75 ppb Kepone and the acutely toxic concentration was 1.0 ppb (TABLE 5).

The sensitivity of C. sapidus larvae to Kepone and to mirex is similar, as might be expected, since mirex is structurally similar to Kepone. Actual concentrations tested revealed 0.1, 0.5 and 0.75 ppb Kepone and 0.01 and 0.1 ppb mirex to be sublethal concentrations and 1.0 ppb Kepone and 1.0 ppb mirex to be acutely toxic concentrations (Bookhout and Costlow, 1975).

There was a marked difference, however, in the sensitivity of R. harrisii larvae to Kepone and to mirex. Concentrations tested revealed 35, 50, 65 and 80 ppb Kepone and 0.01 and 0.1 ppb mirex to be sublethal concentrations, and 95, 110 and 125 ppb Kepone and 1.0 and 10.0 ppb mirex to be acutely toxic concentrations (Bookhout et al., 1972). We have no explanation for R. harrisii larvae being so much more resistant to Kepone than to mirex.

The range of concentrations of Kepone in which development of the decapod, C. sapidus, occurred (TABLE 5) was much more narrow than the range reported for the survival of 48-h-old juvenile Mysidopsis bahia, a mysid, exposed to different concentrations of Kepone for 19 days (Nimmo et al., 1977). Survival of mysids was 90.6% in 0 µg/l, 84.4% in 0.39 µg/l, 50% in 1.55 µg/l, 3.1% in 4.4 µg/l and 0% in 8.7 µg/l. Thus M. bahia was not as sensitive to Kepone as C. sapidus larvae, but much more sensitive than R. harrisii larvae. When adult sheephead minnows, Cyprinodon variegatus, were exposed to Kepone, survival decreased in relation to increase in concentration and duration of exposure. All fish died by day 15 when exposed to 7.8 and

24 µg/l Kepone and 20% died in 0.8 µg/l. The effect of Kepone in water on embryos, fry and juveniles exposed to 0.08, 0.18, 0.72, 6.6 and 32 µg/l was less than that observed when adult fish were exposed to the same concentrations. The 36-day LC50 to developing fish exposed to Kepone in water was 6.7 µg/l (Hansen et al., 1977). Thus the sensitivity of sheepshead minnows to Kepone in chronic bioassays is closer to that found for C. sapidus larvae than for R. harrisii larvae.

Duration

The reduction in molting rate with increase in concentration of Kepone in zoeal development of R. harrisii and in development from hatching to 1st crab in R. harrisii (Figure 3) and C. sapidus is considered a sublethal effect of Kepone (Figure 6). In these species, a similar reduction occurred with each increase in concentration of methoxychlor (Bookhout et al., 1976, malathion (Bookhout and Monroe, 1977) and mirex (Bookhout et al., 1972); however, in mirex the reduction occurred only when C. sapidus was reared in acutely toxic concentrations (Bookhout and Costlow, 1975). Costlow et al. (1960, 1962, 1966) reported that high and low salinities reduce the molting rate of Sesarma cinereum, Panopeus herbstii and Rhithropanopeus harrisii. Thus it is possible that the reduction in molting rate is a response of larvae to any physiological stress and not a direct action of Kepone on the crab's neuro-endocrine system which controls molting.

Seasonal considerations

There is some evidence of seasonal variability in survival and duration of C. sapidus larvae from the time of hatching to the 1st crab stage. Based on published (Bookhout and Costlow, 1975; Bookhout et al., 1976; Bookhout and Monroe, 1977) and unpublished data, it can be hypothesized that C. sapidus larvae hatched in the early part of the breeding season, May to mid-June, at Beaufort, North Carolina, have a higher survival and a shorter duration of development than those reared in the latter part of the breeding season, mid-July to September. According to this concept, a survival of 22.7% and a duration of 54.7 days to 1st crab in acetone control is what might be expected for hatches of C. sapidus at the end of July. It is obvious that there is need for further studies designed to develop a seasonal baseline and also to determine whether there are seasonal biochemical changes in the egg associated with an increase in water temperature.

Mortality

The effect of Kepone on mortality within the range of sensitivity for R. harrisii larvae is shown in Figure 4. The changes in larval mortality by stages and concentrations are consistent with the postulation that as early-stage larval mortality increases with increasing Kepone levels, the remaining larvae at subsequent stages are those most resistant to the insecticide. This process of eliminating larger and larger fractions of susceptible larvae

continues at each increasing concentration of Kepone until a level is reached at which all but the most resistant larvae succumb during the first and second zoeal stages.

The contrast of Figure 4 with Figure 7 shows clearly that larvae of R. harrisii are susceptible to the higher concentrations of Kepone during the first zoeal stage and C. sapidus are not. The results in Figure 7 show significant increases in mortality over the previous stage of zoeal stages II, III and IV in the control and in all concentrations of Kepone. Only at the 1.0 ppb level are mortalities significantly different from the control, but the trend of increasing mortality with concentration is quite consistent with the concentration effects seen in the analysis of survival to megalopa and to 1st crab.

Ecological implications

The presence of Kepone in water, biota and sediments of the Appomatox and James Rivers, Virginia, has been well-documented (Environmental Protection Agency, 1975). It is the consensus that the James River will remain contaminated for years. There may be other contaminated rivers and estuaries, especially outside of the United States, where Kepone is used to control pests that attack bananas and potatoes.

Up to this time there is no direct evidence of toxic effects of Kepone on crab larvae in the James River, the Chesapeake or its tributaries; hence, the potential impact of Kepone on R. harrisii and C. sapidus larvae in the field is inferred only from a few field studies and a number of laboratory investigations on other estuarine organisms.

Rhithropanopeus harrisii larvae are common to abundant in the upper York and Pamunkey Rivers where Sandifer (1973) collected their larvae in a range of salinities from 0 to 21.03^{0}/oo but primarily within a range of 0 to 10^{0}/oo. They were found as early as May and as late as October, but were most numerous from July to September. A similar distribution of R. harrisii larvae in relation to salinity and seasonal abundance in the York River might be expected in the two-layered James River estuary. Since there is a net horizontal flow down the James estuary in the upper layer and a net horizontal flow up the estuary (Prichard, 1953), most R. harrisii larvae should be retained in the middle estuary where salinities are low.

Peak abundance of larvae is where salinities range from 0 to 5^{0}/oo in the York River (Sandifer, 1973); therefore, ovigerous R. harrisii would be expected to be in the same salinities in the James. Thus adults would be in the middle estuary of the James and in adjacent creeks and embayments south of Jamestown.

Since the middle estuary of the James River is known to be polluted with Kepone (Huggett et al., in press), it is reasonable to assume that adult R. harrisii and their eggs are polluted. Newly hatched larvae would have an initial burden of Kepone from eggs, and during development larvae would

acquire additional Kepone from water and food. In the middle estuary, dissolved Kepone is estimated to be in parts per trillion (Water Control Board, 1977; Batelle, 1978; Saleh and Lee, 1978), and phytoplankton, zooplankton and detritus have Kepone residues of 1.30, 4.80 and 0.67 µg/g-dry weight, respectively (Huggett et al., in press). Sediments associated with organic matter are more heavily contaminated with Kepone in the middle estuary than in the lower estuary (Huggett et al., in press).

Bioconcentration of Kepone from water during a developmental period of 16 to 19 days in R. harrisii is not known, but Bahner et al. (1977) reported that it was up to 13,000 times the amount measured in exposure water when mysids were exposed to 0.026 µg/l for 14 days. Epifanio (1973) estimated that if crab larvae were exposed to a field concentration of dieldrin of 2 parts per trillion in seawater and 20 ppb of the pesticide in food, crab larvae would take up the pesticide in 1.23 times as fast from water as from food. Larvae of R. harrisii would obtain an additional burden of Kepone by ingesting zooplankton. In the middle estuary, zooplankton is estimated to have a residue of 4.80 µg/g dry weight (Huggett et al., in press). Kepone once bioconcentrated, is not readily lost, because depuration in crustaceans is slow (Bahner et al., 1977).

The migration of ovigerous Callinectes sapidus from brackish waters to the mouths of estuaries or beyond to spawn has been known for many years (Churchill, 1921). Male blue crabs from the James River had an average Kepone residue of 0.81 µg/g and females 0.19 µg/g. The difference is possibly related to the time spent in the river (Huggett et al., in press). It is very probable that the eggs of the blue crab are also contaminated with Kepone and the body burden of Kepone is in freshly hatched zoeae. Van Engel (1958) found the greatest number of zoeae in channels between Cape Charles and Cape Henry with diminishing numbers up-bay and seaward. Sandifer (1973) collected most zoeae in salinities of 20 to 30°/oo. In the lower Chesapeake and its mouth, Kepone in water, biota and sediments is very much diluted compared to the middle estuary. Larvae of C. sapidus, however, are much more sensitive than the larvae of R. harrisii. Concentrations which were sublethal were 0.1 ppb to 0.75 ppb, and 1.0 ppb was acutely toxic. Furthermore, Provenzano et al. (1978) reported that the female and eggs of grass shrimp, Palaemonetes pugio, collected from the mouth of the James River had Kepone residues of 0.63 and 0.7 ppm, respectively, and samples from Lafayette River in the vicinity of Norfolk had residues of 0.04 and 0.4 ppm, respectively. This is further evidence that the eggs of C. sapidus would be contaminated with Kepone, and from the time of hatching to the 1st crab stage C. sapidus zoeae would obtain additional Kepone from water and zooplankton. Megalopa of C. sapidus might be found up the James River where they would receive the highest concentrations of Kepone of any developing stage. Williams (1971) found that a significant number of C. sapidus megalopa move toward the upper reaches of North Carolina estuaries.

It is obvious that the relationship between Kepone residues and mortality during development of R. harrisii and C. sapidus in the laboratory needs to be determined. It seems reasonably certain that eggs and larvae of R. harrisii in the middle estuary of the James River and the eggs and larvae of C. sapidus in the vicinity of the mouth of the Chesapeake Bay

are polluted with Kepone, but the concentrations are not known. If chemical analysis could be made of eggs in an advanced stage of development, of zoeae, and of megalopa, and compared to residue analyses of similar stages in the laboratory, it should be possible to predict the potential effect of Kepone on populations of the two species.

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GLOSSARY

acute toxicity tests: short-term exposure to concentrations of toxicant which will be lethal to 50% of the larvae in a short interval of time- 24 h, 48 h, or 96 h.

acutely toxic concentrations: concentrations of insecticide in which less than 10% of the larvae survive to the 1st crab stage.

analysis of variance: a special application of the linear models technique which can be used effectively when the experimental design is balanced with respect to factors and replication.

chronic tests: long-term exposure to toxicant.

cummulative mortality: the total number of larval deaths incurred at any given stage of development expressed as a percent of the initial number of larvae.

differential survival: reduction of survival with each increase in insecticide.

dosage-response relationship: the characterization of the change in response (e.g. survival) with changing stimulus (e.g. concentrations of insecticide). Typically such responses vary from 0% at some threshold level of the stimulant to 100% at some uniformly lethal level of the stimulant. An intermediate point is the ED50, the "effective dose" at which 50% of the organisms react to the stimulant.

first crab stage: first stage after molt from megalopa; has adult morphology with abdomen bent under cephatothorax, but is sexually immature.

fitting a linear regression: another special application of the linear models technique where the response variable is a simple linear function of a single independent variable, $y = \alpha+\beta x + \varepsilon$, and the relevant statistics are estimates of the parameters, α, β, and σ^2, the variance of the random error, ε.

general linear models technique: an attempt to characterize a given response (e.g. survival) as a linear function of factors, experimentally imposed and environmentally existent, and their interactions. Statistical analysis of the resulting model quantitatively evaluates the relative importance of the several factors and the experimental errors.

h: hour

LC50: lethal concentration; the concentration of toxicant in water estimated to be lethal to 50 percent of test animals for a specified period of exposure.

megalopa: stage of development of a crab between last zoeal stage and 1st crab stage; is dorso-ventrally depressed; has all cephalothoracic and abdominal appendages present and functional; and has extended abdomen.

µg/g: micrograms per gram = parts per million.

µg/l: micrograms per liter = parts per billion.

mg/l: milligrams per liter = parts per million.

molt: the process of shedding the exoskeleton which is necessary for growth during larval and juvenile development in arthropods, including crustaceans.

ppb: parts per billion.

ppm: parts per million.

$^{o}/_{oo}$ = parts per thousand.

regression coefficient, in the linear regression model: the regression coefficient of the independent variable and the slope of the straight line relating y to x. If y is measured in 'DAYS' and x in 'ppb,' the units of slope are DAYS/ppb.

sublethal concentrations: concentrations of insecticide in which 10% or more of the larvae survive to the 1st crab stage.

sublethal effects: effects in larvae reared in sublethal concentrations, but not in acetone control; they become more pronounced as concentrations are increased.

sub-plot error: the component of experimental error that affects the repeated measurements on the same experimental unit, e.g. cumulative mortality of an original unit of 100 larvae.

technique of split-plot analysis of variance: sometimes called a "repeated measurement design" when successive measurements are taken on the same experimental unit, e.g., survival at each stage of development. The resulting analysis provides for two or more levels at which different components of experimental error may affect the response.

transformed to angular scale: the transformation of data expressed in 'percent' to a new scale where the $\sqrt{\text{percent}}$ is treated as the sine of an angle. While 'percent' varies from 0 to 100 the corresponding 'angles' vary from 0^0 to 90^0. The angular scale is more amenable to statistical analysis because the sampling variance is approximately constant whereas the variance in the percent scale is not.

weighted standard error: a standard error that combines the estimate of error associated with experimental units treated alike (whole-plot error) with the estimate of sub-plot error to provide an appropriate basis for comparing sub-plot means at different levels of whole-plot factors, e.g., to compare the mortality at a given zoeal stage at several different concentrations of insecticide.

zoea(e): a planktotropic larval stage of a crab with a laterally compressed cephalothorax and abdomen, and two thoracic appendages (maxillipeds) for swimming.

zoeal development: refers to all zoeal stages from time of hatching to megalopa stage (i.e., four zoeal stages in R. harrisii and seven to eight zoeal stages in C. sapidus).